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Supporting Information

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Supporting Information for

New aminocyclitols as pharmacological chaperones for glucocerebrosidase, a defective enzyme in Gaucher disease

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Chemistry

General methods: Solvents were distilled prior to use and dried by standard methods. FT-IR spectra are reported in cm^{-1}. ^{1}H and ^{13}CNMR spectra were obtained in $CDCl_3$ solutions at 300 MHz (for ^{1}H) and 75 MHz (for ^{13}C), respectively, unless otherwise indicated. Chemical shifts were reported in delta (δ units, parts per million (ppm) relative to the singlet at 7.24 ppm of $CDCl_3$ for ^{1}H and in ppm relative to the center line of a triplet at 77.0 ppm of $CDCl_3$ for ^{13}C. ESI/HRMS spectra were recorded on a Waters LCT Premier Mass spectrometer.

Compounds **1a, 1b, 1d, 1h, 1o, 2**, **3a, 3b, 3d, 3h, 3o**, **4h**, and **5o** were prepared as described in: M. Egido-Gabas, P. Serrano, J. Casas, A. Llebaria, A. Delgado, *Org. Biomol. Chem.* **2005**, *3*, 1195-1201. NNDNJ was obtained by reductive amination of *tetra*-O-benzyldeoxynojirimycin (D. J. Hardick, D. W. Hutchinsons, S. J. trew, E. M. H. Wellington, *Tetrahedron* **1992**, *48*, 6285-6296), followed by debenzylation with BCl_3.

Aminocyclitols 3c, e, f, g, i, j by reaction of epoxide 2 with amines.

A solution of epoxide **2** (365 mg, 0.7 mmol) in CH_3CN (10 mL) was added dropwise under an argon atmosphere over $LiClO_4$ (2 g, 19 mmol) at rt. A solution of the corresponding amine (5 mmol) in CH_3CN (10 mL) was next added and the reaction mixture stirred at 80º C under argon. After 18 h, the reaction mixture was cooled to rt, quenched with water (10 mL), extracted with dichloromethane (3 × 20 mL) and dried. Filtration and evaporation afforded crude compounds, which were purified by flash chromatography on hexanes:EtOAc (2:1).

3c: (85% yield)

IR: 3497, 3064, 3031, 2945, 2839, 1953, 1870, 1812, 1735, 1496, 1454, 1398.

^{13}CNMR (75 MHz): 14.0, 22.5, 26.9, 29.0, 29.7, 29.5, 31.7, 46.9, 61.8, 75.2-75.9 (OCH_2Ph), 79.7, 82.8, 83.9, 84.9, 127.6-128.5 (CH_{Ar}), 138.1-138.6 (C_{Ar}).

^{1}H NMR (300 MHz): 0.86 (m, 3H, CH_3), 1.20-1.32 (m, 10H, 5 x C*H*$_2$), 2.50-2.79 (m, 3H, H6+NCH_2), 3.43-3.58 (m, 5H, H1-H5), 4.68-4.99 (m, 8H, 4 x OC*H*$_2$Ph), 7.25-7.37 (m, 20H, H_{Ar})

$[a]_D^{25}$ (c 1, $CHCl_3$): -3.8

HRMS: *m/z* calcd.for C41H52NO5 (M+1)**:** 638.3847**;** found: 638.3832

3e: (83% yield)

IR: 3414, 3316, 3105, 3067, 3032, 2964, 2929, 2856, 1490, 1456.

^{13}CNMR (75 MHz): 14.0, 22.6, 27.0, 29.2, 29.4, 29.5, 29.6, 30.7, 31.8, 46.7, 61.7, 75.2-75.8 (OCH_2Ph), 79.9, 82.8, 83.9, 84.9, 127.6-128.5 (CH_{Ar}), 138.1-138.6 (C_{Ar})

^{1}H NMR (300 MHz): 0.82 (m, 3H, CH_3), 1.16-1.28 (m, 14H, 7 x C*H*$_2$), 2.47-2.70 (m, 3H, H6+NCH_2), 3.40-3.58 (m, 5H, H1-H5), 4.61-4.94 (m, 8H, 4 x OC*H*$_2$Ph), 7.22-7.30 (m, 20H, H_{Ar})

$[a]_D^{25}$ (c 1, $CHCl_3$): -4.5

HRMS: *m/z* calcd. for C43H56NO5 (M+1)**:** 666.4160**;** found: 666.4172

3f: (82% yield)

IR: 3414, 3316, 3105, 3067, 3032, 2964, 2929, 2856, 1490, 1456

^{13}CNMR (75 MHz): 14.1, 22.6, 27.1, 29.3, 29.4, 29.5, 29.6, 29.7, 30.7, 31.8, 46.3, 61.6, 75.3-76.5 (OCH_2Ph), 80.3 (C3), 83.0 (C5), 84.0 (C4), 85.1 (C2), 127.6-128.5 (CH_{Ar}), 138.3-138.7 (C_{Ar})

^{1}H NMR (300 MHz): 0.86 (m, 3H, CH_3), 1.22-1.33 (m, 16H, 8 x C*H*$_2$), 2.47-2.60 (m, 3H, H6+NCH_2), 3.39-3.69 (m, 5H, H1-H5), 4.65-4.98 (m, 8H, 4 x OC*H*$_2$Ph), 7.24-7.30 (m, 20H, H_{Ar})

$[a]_D^{25}$ (c 1, $CHCl_3$): -5.4

HRMS: *m/z* calcd. for C44H58NO5 (M+1)**:** 680.4317**;** found: 680.4332

3g: (84% yield)

IR: 3320, 3101, 3074, 3037, 2925, 2852, 1498, 1448

^{13}CNMR (75 MHz): 14.0, 22.6, 26.9, 29.3, 29.4-29.7, 30.7, 31.8, 47.1, 61.9, 75.2-75.9 (OCH_2Ph), 79.5, 82.8, 83.9, 84.9, 127.6-128.5 (CH_{Ar}), 138.0-138.5 (C_{Ar})

^{1}H NMR (300 MHz): 0.89 (m, 3H, CH_3), 1.22-1.33 (m, 28H, 14 x C*H*$_2$), 2.55-2.71 (m, 3H, H6+NCH_2), 3.40-3.66 (m, 5H, H1-H5), 4.67-5.03 (m, 8H, 4 x OC*H*$_2$Ph), 7.26-7.34 (m, 20H, H_{Ar})

$[a]_D^{25}$ (c 1, $CHCl_3$): -1.6

HRMS: *m/z* calcd. for C50H70NO5 (M+1)**:** 764.5256**;** found: 764.5242

3i: (90 % yield)

IR: 3158, 3063, 3028, 2906, 2897, 1493, 1456, 1419

^{13}CNMR (75 MHz): 28.7, 29.6, 35.49, 61.7, 75.2, 75.6, 75.8, 75.9, 82.7, 83.7, 84.7, 126.3-128.5 (CH_{Ar}), 138.2-139.1 (C_{Ar}), 141.9 (C_{Ar})

^{1}H NMR (300 MHz): 1.45 (m, 2H, CH_2), 1.55 (m, 2H, CH_2), 2.55 (m, 3H, H6, CH_2), 2.76 (m, 2H, NCH_2), 3.35-3.81 (m, H1-H5), 4.71-5.01 (m, 8H, 4 x OC*H*$_2$Ph), 7.22-7.39 (m, 25H, H_{Ar})

$[a]_D^{25}$ (c 1, $CHCl_3$): -1.2

HRMS: *m/z* calcd. for C44H50NO5 (M+1)**:** 672.3691**;** found: 672.3677

3j: (90 % yield)

IR: 3373, 2929, 2897, 1496, 1460

^{13}CNMR (75 MHz): 44.1, 51.4, 52.3, 61.8, 75.0-75.8 (OCH_2Ph), 80.7, 82.9, 83.9, 84.9, 126.4-128.5 (CH_{Ar}), 138.2-144.8 (C_{Ar})

^{1}H NMR (300 MHz): 2.55 (t, J=10.2 Hz, 1H, H6), 3.30-3.65 (m, 7H, H1-H5, NCH_2), 4.03 (t, J=7.5Hz, 1H, $CHPh_2$), 4.63-5.01 (m, 8H, 4 x OC*H*$_2$Ph), 7.19-7.39 (m, 30H, H_{Ar})

$[a]_D^{25}$ (c 1, $CHCl_3$): -2.2

HRMS: *m/z* calcd. for C48H50NO5 (M+1)**:** 720.3691; found: 720.3702

3k: (73 % yield)

IR: 3302, 3075, 3051, 3027, 1490, 1456, 1450

^{13}CNMR (75 MHz): 36.5, 44.7, 48.9, 61.6, 75.1-75.9 (OCH_2Ph), 80.3, 82.9, 83.9, 84.9, 126.1-128.5 (CH_{Ar}), 138.3-144.5 (C_{Ar})

^{1}H NMR (300 MHz): 2.16 (m, 2H, CH_2), 2.47 (t, J=10.2 Hz, 2H, NCH_2), 2.64 (m, 1H, H6), 3.34-3.62 (m, 5H, H1-H5), 3.95 (t, J=7.8 Hz, 1H, $CHPh_2$), 4.56-4.97 (m, 8H, 4 x OC*H*$_2$Ph), 7.18-7.41 (m, 30H, H_{Ar})

$[a]_D^{25}$ (c 1, $CHCl_3$): -4.4

HRMS: *m/z* calcd. for C49H52NO5 (M+1)**:** 734.3847; found: 734.3831

Reductive amination. Synthesis of 5l-n by reductive amination of 3d and 3h

A solution of the starting amino alcohol **3d** or **3h** (0.05 mmol) in MeOH (2 mL) under an atmosphere of argon was treated successively with sodium cyanoborohydride (4.2 mg, 0.108 mmol), acetic acid (3.5 μL) and the aldehyde (0.05 mmol). After stirring for 18 h at rt, the mixture was quenched with water (2 cm3) and extracted with diethyl ether (3 × 20 cm3). The combined organic layers were washed with brine, dried and concentrated under a reduced pressure to afford crude amino alcohols, which were purified by flash chromatography using a mixture of dichloromethane–methanol (12: 1).

5l (87 % yield from **3d**)

^{13}CNMR (75 MHz): 14.3, 22.7, 27.5, 29.3, 29.4, 29.7, 30.5, 32.1, 65.0, 70.2, 74.4, 75.3, 75.9, 76.0, 79.2, 83.1, 84.5, 86.0, 127.6-128.5 (CH_{Ar}), 138.3-138.7 (C_{Ar})

^{1}H NMR (300 MHz): 0.91 (t, J=6.5 Hz, 6H, 2 x CH_3), 1.25-1.56 (m, 24H, 12 x C*H*$_2$), 2.30-2.65 (m, 5H, H6+2 x NCH_2), 3.33-3.83 (m, 5H, H1-H5), 4.71-5.06 (m, 8H, 4 x OC*H*$_2$Ph), 7.25-7.43 (m, 20H, H_{Ar})

HRMS: *m/z* calcd. for C50H70NO5 (M+1)**:** 764.5256; found: 764.5285

5m (91% from **3d**)

^{13}CNMR (75 MHz): 14.4, 22.9, 27.6, 29.4, 29.5, 29.6, 29.8, 30.3, 32.1, 64.9, 70.4, 74.5, 75.3, 76.0, 76.2, 79.1, 83.4, 84.5, 86.1, 124.0-128.6 (CH_{Ar}), 136.0-140.7 (C_{Ar})

^{1}H NMR (300 MHz): 0.94 (t, J=6.5 Hz, 3H, CH_3), 1.26-1.34 (m, 12H, 6 x C*H*$_2$), 1.52 (m, 2H, CH_2), 2.60-2.90 (m, 5H, H6 + 2 xNCH_2), 3.32-3.67 (m, 5H, H1-H5), 4.541-5.05 (m, 8H, OCH_2Ph), 7.25-7.47 (m, 25H, H_{Ar})

HRMS: *m/z* calcd. for C50H62NO5 (M+1)**:** 756.4630; found: 756.4610

5n (91 %yield from **3h**)

IR: 3438, 3062, 3030, 2917, 1497, 1457, 1363

^{13}CNMR (75 MHz): 29.6, 36.3, 64.4, 70.3, 74.2, 74.9, 75.7, 75.9, 78.8, 82.6, 83.9, 85.5, 127.4-128.6 (CH_{Ar}), 138.3-139.8 (C_{Ar})

^{1}H NMR (300 MHz): 2.66 (m, 1H, H6), 2.82-2.95 (m, 8H, CH_2CH_2), 3.26-3.62 (m, 5H, H1-H5), 4.66-4.99 (m, 8H, 4 x OC*H*$_2$Ph), 7.13-7.34 (m, 30H, H_{Ar})

HRMS: *m/z* calcd. for C50H54NO5 (M+1)**:** 748.4004; found: 748.4032

Aminocyclitols 1 by debenzylation with BCl_3

A solution of 0.05 mmol of the starting benzylated amino alcohol in dichloromethane (2 mL) under at atmosphere of nitrogen at –78ºC, was treated with 2.5 eq per benzyl group of a 1*M* solution of boron trichloride in heptane. After stirring for 2 h at –78ºC, the reaction mixture was allowed to warm to 0ºC and stirred for additional 24 h. The mixture was cooled to -78ºC, quenched with methanol (1 mL) and evaporated under reduced pressure. The resulting residue was suspended in $CHCl_3$ and stirred vigorously at rt for 30 min. The mixture was filtered and the solid washed thoroughly with $CHCl_3$. The residue was taken up in MeOH (HPLC grade) and evaporated to dryness to afford the final aminocyclitol as the corresponding hydrochloride salt in quantitative yield.

1c (HCl):

^{1}H NMR (300 MHz, CD_3OD): 0.89 (m, 3H), 1.30-1.36 (m, 8H), 1.70 (m, 2H), 3.01 (t, J=6.0Hz, 1H), 3.05-3.20 (m, 3H), 3.25 (t, J=5Hz, 2H), 3.55 (t, J=6.3 Hz, 2H).

^{13}CNMR (75 MHz, CD_3OD): 13.4, 22.6, 26.4, 26.6, 28.8, 31.6, 45.4, 62.8, 70.2, 74.9, 76.4.

HRMS, *m/z* calcd. for C13H28NO5 (M+1)**:** 278.1969**;** found: 278,1977;

1e (HCl)

^{1}H NMR (300 MHz): 0.91 (m, 3H), 1.32-1.35 (m, 12H), 1.73 (m, 2H), 2.98 (t, J=6.5Hz, 1H), 3.10-3.25 (m, 3H), 3.28 (t, J=5Hz, 2H), 3.52 (t, J=6.3 Hz, 2H).

^{13}CNMR (75 MHz, CD_3OD): 13.4, 22.7, 26.3, 26.6, 29.2, 29.3, 29.5, 31.9, 45.3, 61.9, 69.1, 74.1, 75.6.

HRMS, *m/z* calcd. for C15H32NO5 (M+1)**:** 306.2282; found: 306.2276;

1f (HCl)

^{1}H NMR (300 MHz): 0.90 (m, 3H), 1.30-1.37 (m, 14H), 1.75 (m, 2H), 2.98 (t, J=6.3Hz, 1H), 3.05-3.20 (m, 3H), 3.25 (t, J=5Hz, 2H), 3.53 (t, J=6.3 Hz, 2H).

^{13}CNMR (75 MHz, CD_3OD): 14.3, 23.6, 27.2, 27.5, 30.1, 30.3, 30.4, 30.5, 32.9, 45.9, 62.7, 69.9, 75.1, 76.5.

HRMS, *m/z* calcd. for C16H34NO5 (M+1)**:** 320.2439; found: 320.2429

1g (HCl)

^{1}H NMR (300 MHz): 0.91 (m, 3H), 1.29-1.36 (m, 26H), 1.74 (m, 2H), 2.97 (t, J=6.0Hz, 1H), 3.14-3.20 (m, 3H), 3.25 (t, J=5Hz, 2H), 3.51 (t, J=6.0 Hz, 2H).

^{13}CNMR (75 MHz, CD_3OD): 14.3, 23.6, 27.6, 30.1-30.7 (11C, alkyl chain), 32.9, 46.0, 62.8, 70.2, 75.1, 76.6.

HRMS, *m/z* calcd. for C22H46NO5 (M+1)**:** 404.3378; found: 404.3390.

1i (HCl)

^{1}H NMR (300 MHz):1.40 (m, 2H), 1.65 (m, 2H), 2.53 (m, 2H), 3.05 (m, 2H), 3.45 (t, J=6.6 Hz, 1H), 3.50-3.65 (m, 3H), 3.67-3.72 (m, 2H), 7.22-7.28 (m, 5H).

^{13}CNMR (75 MHz, CD_3OD): 26.7, 29.4, 36.1, 46.1, 49.9, 62.9, 70.1, 75.1, 76.5, 126.9, 129.1, 129.5, 142.8.

HRMS, *m/z* calcd. for C16H26NO5 (M+1)**:** 312.1813; found: 312.1819.

1j (HCl)

^{1}H NMR (300 MHz): 3.08 (t, J=6.6Hz, 1H), 3.17 (m, 2H), 3.58 (m, 2H), 3.75 (m, 1H), 3.92 (m, 2H), 4.52 (m, 1H), 7.28-7.47 (m, 10H).

^{13}CNMR (75 MHz, CD_3OD): 49.5, 49.8, 63.6, 69.9, 75.2, 76.6, 128.6, 129.0, 130.2, 141,2.

HRMS, *m/z* calcd. for C20H26NO5 (M+1)**:** 360.1813; found: 360.1805.

1k (HCl)

^{1}H NMR (300 MHz): 2.48 (m, 2H), 3.14 (m, 2H), 3.27 (broad t, 1H), 3.32 (broad, 3H), 3.46 (m, 1H), 3.59 (broad t, 1H), 4.08 (broad t, 1H), 7.14-7.45 (m, 10H).

^{13}CNMR (75 MHz, CD_3OD): 32.8, 46.0, 49.8, 63.3, 70.4, 74.9, 76.4, 127.6, 128.7, 129.7.

HRMS, *m/z* calcd. for C21H28NO5 (M+1)**:** 374.1969; found: 374.1984.

1l (HCl)

^{1}H NMR (300 MHz): 0.87 (m, 6H), 1.27-1.39 (m, 24H), 2.88 (m, 4H), 3.25 (t, J=6Hz, 1H), 3.46 (m, 3H), 3.52 (m, 2H).

^{13}CNMR (75 MHz, CD_3OD): 13.2, 23.1, 26.4, 27.2, 28.8, 29.0, 31.9, 47.2, 60.5, 68.4, 73.7, 75.8.

HRMS, *m/z* calcd. for C22H46NO5 (M+1)**:** 404.3378; found: 404.3372.

1m (HCl)

^{1}H NMR (300 MHz): 0.89 (t, 3H), 1.12-1.28 (m, 10H), 1.66 (broad, 2H), 3.10-3.75 (m, 11H), 3.96 (broad, 1H); 7.28 (m, 5H).

^{13}CNMR (75 MHz, CD_3OD): 14.4, 23.7, 26.2, 27.4, 30.1, 30.2, 32.1, 32.9, 53.9, 56.1, 65.8, 69.2, 74.9, 76.5, 128.5, 130.1, 130.3, 137.3.

HRMS, *m/z* calcd. for C22H38NO5 (M+1)**:** 396.2752**;** found: 396.2764.

1n (HCl)

^{1}H NMR (300 MHz): 3.07 (broad, 4H), 3.23-3.37 (m, 4H), 3.56 (m, 3H), 3.67 (m, 1H), 3.92 (broad, 2H), 7.26 (m, 10H)

^{13}CNMR (75 MHz, CD_3OD): 32.2, 55.5, 65.9, 69.3, 75.0, 76.6, 128.5, 130.1 (2x), 137.3

HRMS, *m/z* calcd. for C22H30NO5 (M+1)**:** 388.2126; found: 388.2119

Enzyme inhibition

Enzyme assays (Table 1)

p-Nitrophenyl glucosides, 4-methylumbelliferyl-β-D-glucoside, ceramide and UDP-glucose were obtained from Sigma. [glucose-^{14}C(U)] UDP-glucose (303 mCi/mmol) was from New England Nuclear. α-Glucosidase (baker's yeast, G5003) and β-glucosidase (almonds, G0395), were purchased from Sigma. Imiglucerase (Cerezyme®) was kindly provided by Genzyme.

Commercial glucosidase solutions were prepared with the appropriate buffer and incubated in 96-well plates at 37ºC without (control) or with inhibitor (1 mM) for 5 minutes. After addition of the corresponding substrate solution, incubations were maintained during 3 minutes and stopped by addition of 1M Tris solution. The amount of *p*-nitrophenol formed was determined at 405 nm with UV/VIS Lector Spectramax Plus (Molecular Devices Corporation) spectrophotometer. α-glucosidase activity was determined with 1mM *p*-nitrophenyl-α-D-glucopyranoside in 100mM sodium phosphate buffer (pH 7.2). β-glucosidase activity was determined with 1mM *p*-nitrophenyl-β-D-glucopyranoside in 100mM sodium acetate buffer (pH 5.0).

Glucocerebrosidase: Lysosomal glucocerebrosidase activity in rat liver membranes suspensions (according to H. S. Overkleeft, G. H. Renkema, J. Neele, P. Vianello, I. O. Hung, A. Strijland, A. M. van der Burg, G. J. Koomen, U. K. Pandit, J. M. Aerts, *J. Biol. Chem.* **1998**, *273*, 26522-26527) was determined with 1mM 4-methylumbelliferyl-β-D-glucopyranoside in McIlvaine buffer (100mM sodium citrate and 200mM sodium phosphate

buffer, pH 5.2). Imiglucerase activity was determined with 2.4mM 4-methylumbelliferyl-β-D-glucopyranoside in the presence of 0.25% (w/v) sodium taurocholate and 0.1% (v/v) Triton X-100 in McIlvaine buffer (pH 5.2). Enzyme solutions were incubated at 37ºC without (control) or with inhibitor (1 mM) during 30 minutes and, after addition of corresponding substrate solution, incubations were maintained at 37ºC for 30 minutes (lysosomal glucocerebrosidase) or 10 minutes (Imiglucerase). Enzymatic reactions were stopped by the addition of 100mM glycine/NaOH buffer (pH 10.6). The amount of 4-methylumbelliferone formed was determined with 1420 VICTOR^2 Multilabel Counter (Wallac) fluorometer at 355nm (excitation) and 460nm (emission).

Lysosomal a-glucosidase. Pig liver lysosomes were incubated with 1mM 4-methylumbelliferyl-a-D-glucopyranoside in acetate buffer (pH 4) at 37ºC without (control) or with inhibitor (1 mM) during 15 minutes. Enzymatic reaction was stopped by the addition of 100mM glycine/NaOH buffer (pH 10.6). The amount of 4-methylumbelliferone formed was determined with 1420 VICTOR^2 Multilabel Counter (Wallac) fluorometer at 355nm (excitation) and 460nm (emission).

Glucosylceramide synthase: Liver microsomes were obtained from Sprage-Dawley rats as described in: P. Paul, Y. Kamisaka, D. L. Marks, R. E. Pagano, J. Biol. Chem. 1996, 271, 2287-2293. Microsomal protein was incubated with 0.12 mM of N-octanoyl-D-sphingosine-BSA complex and 1.6 mM NAD in 50mM TRIS-HCl buffer (pH 7.4) with or without (control) 0.5 mM inhibitor for 10 minutes at 37ºC. After addition of 0.04 mM [^{14}C]UDP-glucose (2 mCi/mmol) incubations were prolonged for 35 minutes and stopped by the addition of 0.5ml $CHCl_3$:CH_3OH (2:1). The [^{14}C]-glucosyl-*N*-octanoyl-D-sphingosine formed was extracted (according to F. M. Platt, G. R. Neises, G. B. Karlsson, R. A. Dwek, T. D. Butters, *J. Biol. Chem.* **1994**, *269*, 27108-27114) and quantified in Wallac 1410 (Pharmacia) liquid scintillation counter.

Inhibition parameters. The IC_{50} values were determined by plotting percent activity versus log [I], using at least five different inhibitor concentrations. Type of inhibition and K_i values for more active inhibitors were determined by Lineweaver-Burk or Dixon plots of assays performed with different concentrations of inhibitor and substrate.

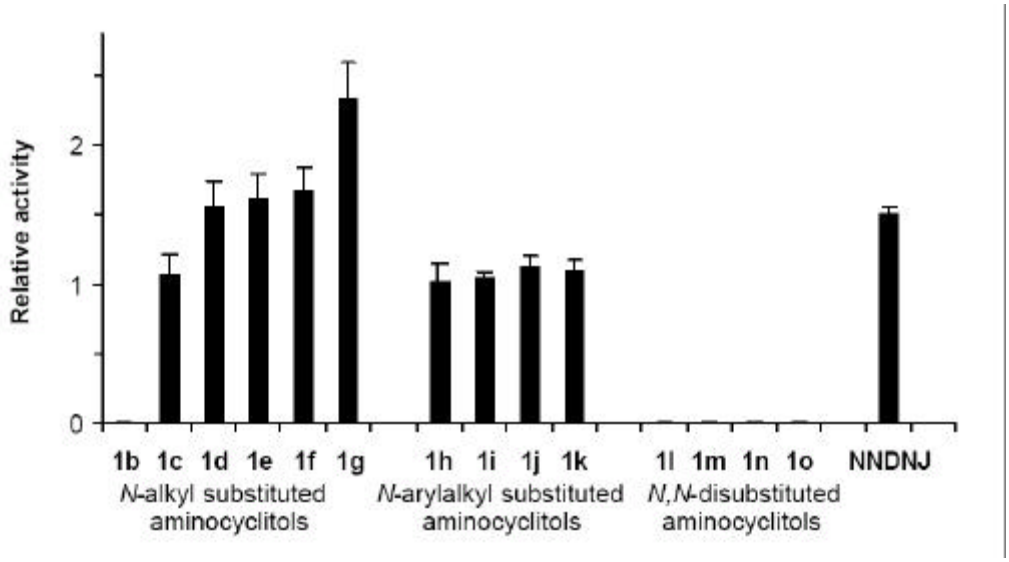
Table 1. Inhibition of different enzymes by aminocyclitols 1a-1o

	Lysosomal GlcCerase		Lysosomal a-glucosidase	GlcCer synthase	a-glucosidase (yeast)	ß-glucosidase (almond)
Compound	**% activity (1mM)**	**IC_{50} (µM)**	**% activity (1mM)**	**% activity (1mM)(a)**	**% activity (1mM) (a)**	**% activity (1mM)(a)**
1a	84	----	94	95	83	91
1b	65	----	94	118	33 (254)	58 (1144)
1c	21	333	97	142	74	81
1d	24	352	93	115	80	88
1e	11	65.6	94	106	76	87
1f	8	12.1	93	91	72	85
1g	6	6.3	92	105	80	86
1h	52	----	93	83	63	80
1i	10	23.1	87	19 (880)	88	89
1j	11	55.8	94	63 (1117)	100	77
1k	9	21.6	93	2 (580)	85	71
1l	12	101	91	14 (139)	100	95
1m	14	182	94	104	100	93
1n	11	184	94	121	88	97
1o	40	350	91	136	93	93

(a) IC_{50} (µM) in brackets

Chaperone assay

Imiglucerase aliquots (48 µl, 2 mg/ml) were incubated with 0, 50, 100, or 150 µM chemical chaperone at 48°C. Subsequently, 150 µl of 0.1 M acetate-phosphate buffer (pH 5.0) and 100 µl of substrate (4 mM 4-methyllumbelliferyl ß-D-glucoside) in McIlvaine buffer (pH 5.2) were added at different times and incubated for 10 min at 37°C, in the presence of 0.l % Triton X-100 and 0.2% taurodeoxycholic acid. Then, 300 µl of glycine buffer (100 mM, pH 10.6) were added and liberated 4-methylumbelliferone was measured. Enzyme activity was reported relative to unheated enzyme. Alternatively, Imiglucerase aliquots (23 µl, 4 mg/ml) were incubated with 0, 50, 100, or 150 µM chemical chaperone in the presence of 25 µl of urea (16 M) for 1 hour. Then, enzyme activity was determined as described above. Results from chaperone assay under urea-induced denaturation are shown below.



Chaperone effect at 100 µM of aminocyclitols **1a-1o** and **NNDNJ** after urea-induced denaturation

Cytotoxicity assays

A549 Cells were obtained from the American Type Culture Collection (ATCC) and grown in HAM F12 with glutamine medium supplemented with 10% fetal bovine serum (FBS). Cells were incubated at 37 ºC in 5% CO_2/95% air for 3 days in the presence of compounds. The number of viable cells was quantified by the estimation of its dehydrogenase activity, which reduces 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to water insoluble formazan, which was dissolved in DMSO and measured at 570 nm with a Multiskan plate reader (Labsystems).